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Persistent carry-over effects of planktonic exposure to ocean acidification in the Olympia oyster

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Abstract. Predicting impacts of global environmental change is challenging due to the complex life cycles that characterize many terrestrial and aquatic taxa. Different life stages often interact with the physical environment in distinct ways, and a growing body of work suggests that stresses experienced during one life stage can "carry over" to influence subsequent stages. Assessments of population responses to environmental perturbation must therefore consider how effects might propagate across life-history transitions. We investigated consequences of ocean acidification (decreased pH and carbonate saturation) for early life stages of the Olympia oyster (*Ostrea lurida*), a foundation species in estuaries along the Pacific coast of North America. We reared oysters at three levels of seawater pH, including a control (8.0) and two additional levels (7.9 and 7.8). Oysters were cultured through their planktonic larval period to metamorphosis and into early juvenile life. Larvae reared under pH 7.8 exhibited a 15% decrease in larval shell growth rate, and a 7% decrease in shell area at settlement, compared to larvae reared under control conditions. Impacts were even more pronounced a week after settlement, with juveniles that had been reared as larvae under reduced pH exhibiting a 41% decrease in shell growth rate. Importantly, the latter effect arose regardless of the pH level the oysters experienced as juveniles, indicating a strong carry-over effect from the larval phase. Adverse impacts of early exposure to low pH persisted for at least 1.5 months after juveniles were transferred to a common environment. Overall, our results suggest that a stringent focus on a single phase of the life cycle (e.g., one perceived as the "weakest link"), may neglect critical impacts that can be transferred across life stages in taxa with complex life histories.

Key words: carry-over effect, complex life cycle, juveniles, larvae, ocean acidification, Ostrea lurida

INTRODUCTION

Anthropogenic increases in greenhouse-gas production and land use alteration are driving dramatic environmental changes in terrestrial and aquatic habitats. Many studies have examined ensuing effects on organismal- and population-level processes, and considerable effort is being devoted to projecting future consequences to natural populations (Parmesan 2006, Helmuth et al. 2006). Attempts at prediction, however, are complicated in many species by the existence of complex life cycles, which result in sequential stages (e.g., larvae, juveniles, adults) that exhibit profoundly different characteristics. Differences include disparate morphologies, physiologies, behaviors, habitats, and species interactions, which in turn can induce unique responses to environmental shifts. Such life cycles are common in a wide variety of terrestrial and aquatic taxa (Pechenik 2010).

A common approach for determining responses of a species with a complex life cycle to global change has been to seek the single stage that is most vulnerable to stress (e.g., Bhaud et al. 1995, Hobbie and Chapin 1998, Previtali et al. 2010). However, while focusing on a putative "weak link" has utility, it also has the potential to neglect effects transferred across stages. In particular, the process of metamorphosis from larva to juvenile usually includes a dramatic remodeling of body form that is energetically expensive. Consequently, juvenile performance (and perhaps even adult fitness) can be impaired by a stressful larval experience that incurs metabolic cost and causes subsequent stages to begin with energetic deficits (Pechenik et al. 1998, Giménez 2010). Poor nutrition, exposure to environmental toxins, salinity stress, and other factors have all been shown to induce negative "carry-over effects" that persist into later stages in a range of taxa (amphibians: Semlitsch et al. 1988, Álvarez and Nicieza 2002; insects: Peckarsky et al. 2001; marine invertebrates: Pechenik et al. 1998, Phillips 2002, Marshall et al.

2003, Emlet and Sadro 2006; fish: Hamilton et al. 2008). Yet, despite the propensity for many species to possess complex life histories, and a demonstrated ability for larval condition to influence later stages, only a handful of recent studies have tested for the presence of carry-over effects in the context of global environmental change (Parker et al. 2012, Dupont et al. 2012).

The present study explores these issues in the Olympia oyster, *Ostrea lurida*, a marine bivalve with a complex life history that includes a planktonic larval stage followed by benthic juvenile and adult phases. Historically, the Olympia oyster was an important habitat-forming species in bays and estuaries along the Pacific coast from Alaska to Baja California, Mexico. Where abundant, it increased local diversity through the provision of hard substrate (Kimbro and Grosholz 2006, Polson and Zacherl 2009), and contributed to the maintenance of water quality through filter feeding (Officer et al. 1982). Since the mid-1800's, however, populations of this species have declined dramatically in California estuaries due to a variety of anthropogenic impacts, and have not recovered despite recent efforts at restoration (Baker 1995, White et al. 2009).

Large-scale changes in seawater chemistry may exacerbate local threats to the Olympia oyster (and indeed, to oysters in general; Miller et al. 2009, Talmage and Gobler 2009, Parker et al. 2009, Waldbusser et al. 2011, Barton et al. 2012). Anthropogenic carbon dioxide not only alters global temperatures, but also modifies the marine carbonate system, causing ocean acidification, characterized by decreased pH and carbonate ion concentration, [CO_3^{2-}] (Caldeira and Wickett 2003). pH levels in the surface ocean have already decreased 0.1 unit since pre-industrial times, and a further 0.3–0.4 unit decrease is expected by 2100 (Feely et al. 2009). Similarly, [CO_3^{2-}] has declined by up to 16% in some coastal waters (Feely et al. 2009), and declines of another 30–35% are predicted by the end of the century (IPCC 2007). These chemical

changes are known to hinder calcification, growth, survival, and reproduction of many marine organisms (Doney et al. 2009). However, few studies have explicitly addressed the transference of effects of ocean acidification between sequential life stages, and in those limited studies effects have been varied. Parker et al. (2012) found that Sydney rock oysters held as adults in elevated pCO_2 conditions for 5 months produced larvae that were more resilient to acidified conditions. In particular, larvae were larger, developed faster, and survived at a similar rate relative to those produced by control oysters. In contrast, Dupont et al. (2012) reported that exposure of adult green sea urchins to elevated pCO_2 conditions for 4 months led to larvae with lower settlement success, while exposure of the larvae themselves to elevated pCO_2 had little effect on settlement success or juvenile growth following metamorphosis.

We investigated whether ocean acidification affects the planktonic larvae of the Olympia oyster, and whether these impacts are transferred across the transition associated with settlement and metamorphosis. We focused on three primary questions: (1) Are there negative effects of reduced pH (elevated CO_2) on larval shell growth rate, (2) do these effects carry over to affect the benthic juvenile stage, and (3) if they carry over, how long might effects persist into early juvenile life? Our results contribute to a nascent but growing awareness that understanding influences of global environmental change on organisms with complex life cycles will benefit from approaches that explicitly address how ecological impacts are transmitted across life stages.

METHODS

General approach

Experimental system – Larval and juvenile oysters were reared at the Bodega Marine Laboratory (BML), Bodega Bay, California in two distinct experiments. Seawater CO_2 concentrations in treatment cultures were increased relative to present-day levels by 100 and 400

ppm, within the range of projected shifts (IPCC 2007). Note that CO_2 levels in estuaries that support Olympia oysters can be strongly elevated during extreme conditions. For example, hydrographic data from our study region (Tomales Bay, California: 38°06'57.01"N, 122°51'14.39"W) indicate CO_2 concentrations from <200 to >1500 ppm, with median values exceeding 700 ppm during summer months when oyster larvae are in the water column (Russell et al., unpubl. data). Consistent with such trends, and recognizing that these extreme conditions can be expected to become more common in the face of global environmental change, seawater CO_2 levels of 700 (used as an operational control), 800, and 1100 ppm were employed in our experiments. Given typical local values of salinity (~34 psu), temperature (~20°C during the reproductive season), and alkalinity (~2230 μmol per kg of seawater, denoted henceforth as $\mu mol/kg_{SW}$), such CO_2 levels correspond to pH values of approximately 8.0, 7.9, and 7.8 when quantified on the NBS scale (Zeebe and Wolf-Gladrow 2001). These nominal pH levels are used subsequently to identify the treatments, with the caveat that impacts could accrue from associated CO_2 shifts, reductions in carbonate saturation, and/or altered pH itself.

Oysters were reared from early larval life in 4.5 L glass culture jars held in seawater tables maintained at 20.0 (±0.02) °C. All seawater used during rearing was filtered at 0.45 μm and pre-adjusted to appropriate pH levels in 20 L carboys by bubbling for 2–3 days with NIST-traceable CO_2 air mixtures (hereafter referred to as "carboy water"). Acrylic boxes mounted over each seawater table received the same mixed gases and provided a common head space for six jars, minimizing off-gassing during culturing. All biological processes in each of the jars and any resultant effects on seawater chemistry, however, operated independently. pH levels used for each box, and jar position within a box, were randomly assigned. A low-rpm electric motor drove oscillating paddles in each jar to provide gentle stirring (Strathmann 1987).

Initiation and maintenance of larval cultures – Adult Olympia oysters (4–7 cm long) were collected from Tomales Bay, transported to BML, cleaned of all epiphytes, and distributed among multiple 100 L cylinders (25 adults/cylinder) containing seawater filtered at 0.45 μm and held at 18–22°C. Female Olympia oysters brood their young within the mantle cavity for 10 days post-fertilization, and at least one female in a given cylinder typically released larvae within 48 hours post-collection. This enabled acquisition of independent "larval cohorts" from different sets of parents for simultaneous initiation of cultures (larvae in a cohort were those produced by one or more females associated with a particular cylinder). Following release, larvae (1000 or 900 in Experiments 1 and 2, respectively) were transferred into each culture jar containing 2 L of seawater filtered at 0.45 μm (day 1 of the experiment). Every other day, 90% of the seawater in each jar was removed using reverse filtration through a 125 μm mesh (hereafter referred to as "jar water"), and replaced with carboy water, whose pH had stabilized at the appropriate level. Immediately following each water change, microalgal food (*Isochrysis galbana*) was added to each jar at a density of 100,000 cells/mL, a level known to encourage rapid growth and high survival of larval and juvenile oysters (Strathmann 1987). This microalgal food density was maintained in the jars throughout each experiment, for both larval and juvenile life stages.

Sampling of water chemistry, larvae, and juveniles – Samples of jar water and carboy water were collected every other day during each water change. Seawater pH (NBS scale) and temperature were quantified using a pH/temperature meter (Accumet Excel XL60), and salinity was determined using a YSI 6600V2 multi-parameter instrument. Alkalinity was measured using automated Gran titration (Metrohm 809), and standardized using certified reference material from A. Dickson at Scripps Institution of Oceanography. Other carbonate system parameters were calculated using the software, CO2SYS (Lewis and Wallace 1998), employing pH_{NBS} and

alkalinity as the primary input variables, equilibrium constants K_1 and K_2 from Mehrbach et al. 1973, and K_{SO4} from Dickson 1990.

Oysters in the culture jars were sampled at key time points during each experiment to quantify shell size and growth rate (change in shell area per day). On day 1, 100 larvae per larval cohort were collected haphazardly by pipette, fixed in 95% ethanol, and individually photographed under a microscope (Leica DM1000 with DC290 camera) for analysis using ImageJ software (ver. 1.37, National Institutes of Health) to determine the initial projected area of the shell. Larval shell growth rates at later time points were calculated similarly, as the increase in projected shell area per day since larval release. Juvenile shell growth rate following settlement was determined by measuring the projected shell area of settled individuals from photographs, subtracting the area of the larval shell (which remains visually distinct), and dividing that value by the number of days post-settlement (Appendix A).

Study elements

Experiment 1 – In the first experiment (July–September 2009), oyster larvae from four larval cohorts were reared in pH 8.0, 7.9, and 7.8 seawater through the duration of planktonic larval development, and for 52 days post-settlement. Each of three pH levels were replicated by two boxes, and all four larval cohorts were represented by 1–2 culture jars in each box (n=36 jars total). Although the inclusion of larval cohort in the experiment originated from a general interest in whether offspring produced by different mothers might respond differently to ocean acidification, a rigorous exploration of this issue would require a controlled breeding design and a quantitative genetic approach (e.g., Sunday et al. 2011) that is beyond the scope of our study. We therefore confined ourselves to the more limited question of whether the four specific larval cohorts in our experiment exhibited different responses to ocean acidification. This more

constrained exploration provides preliminary insight into whether variation among larval cohorts could be relevant for understanding consequences of altered seawater chemistry.

Fifteen larvae were haphazardly sampled from each jar at day 9 and fixed in 95% ethanol for later photographing. Larvae were allowed to settle directly onto the bottoms of the jars. Larvae reached competency on approximately day 11, and by day 13, the majority of larvae had settled across all treatments. Seven days after settlement, the bottom of each jar was removed and juveniles attached to the jar bottoms were photographed (20 per jar). The juveniles were then transferred to a flow-through seawater table maintained at 20^{o}C, where they received ambient food concentrations from Bodega Bay. After 45 days in this common garden environment (52 days after settlement), juveniles were again photographed to determine their size.

Experiment 2 – Reduced pH induced a negative effect on juvenile growth rate (see *Results*). This outcome could have arisen from three causes: direct effects of seawater acidification during the early juvenile phase, transition shock tied to newly settled individuals moving from treatment to normal seawater, or from carry-over effects of larval experience. Experiment 2 (September–October 2009) was conducted to distinguish among these alternatives. In the first phase of Experiment 2, larvae were pooled across cohorts (300 larvae/cohort; 900 larvae/replicate jar) and reared through settlement under either control or low pH conditions (pH 8.0 or 7.8). pH levels were randomly assigned to four boxes (2 pH levels x 2 boxes x 3 replicate jars = 12 jars). In the second phase of the experiment, and within 24 hrs of larval settlement, one half of the juveniles reared as larvae in control pH conditions were transferred to low pH conditions (denoted 8.0→7.8), and one half were returned to control pH conditions (denoted 8.0→8.0) (Fig. 1). Similarly, one half of the juveniles that had been reared as larvae in low pH conditions were transferred to control pH conditions (7.8→8.0), and one half were returned to

low pH conditions (7.8→7.8). These transfers were accomplished by allowing larvae to settle on the bottoms of the culture jars, removing the bottoms, photographing juveniles to quantify shell area at settlement (17 per jar), then cutting the jar bottoms into four wedges and allocating two of the four wedges from each jar to new jars maintained at the appropriate pH level. The second phase of the experiment used 2 pH levels x 4 larval-juvenile pH treatments x 6 replicate jars = 24 jars. The juveniles in the two destination pH levels were reared until 13 days after settlement, and randomly selected juveniles on wedges (17 per jar) were photographed 7 and 13 days after settlement to quantify shell growth rate since settlement. Larval settlement occurred 24 hrs earlier (day 13) under low pH conditions; therefore all photographs taken of settled juveniles in control conditions were delayed by 1 day.

We anticipated three potential outcomes for Experiment 2 (Fig. 1). First, if impacts of low pH on juvenile shell growth rate were driven by greater sensitivity of juveniles, then shell growth rates would be lowest among individuals raised as juveniles under reduced pH, regardless of earlier larval experience (termed the "Juvenile sensitivity hypothesis"). Second, if low juvenile growth were caused by the shock of being transferred to seawater with different carbonate chemistry, then individuals in the 8.0→7.8 or 7.8→8.0 treatments would exhibit the lowest shell growth ("Switching hypothesis"). Third, if the negative effects on juvenile shell growth were driven by carry-over effects initiated at the larval stage, then juvenile shell growth would be lowest among individuals that began life in the low-pH conditions (i.e., 7.8→7.8 and 7.8→8.0), regardless of the pH conditions in which the juveniles were raised ("Larval carry-over hypothesis").

Statistical analyses

In Experiment 1, larval shell growth rate, shell area at settlement, and juvenile shell growth rate were analyzed using separate, partly nested ANOVAs with jar means as replicates. In this split-plot design, pH level was the whole-plot factor, larval cohort was the sub-plot factor, and boxes were nested within pH level. Larval cohort was set as a fixed factor in our analyses to avoid overly broad inferences based on a small number of cohorts. The water chemistry data were analyzed using this same ANOVA structure, and separate ANOVAs were conducted for pH and alkalinity. For each jar, all measurements of jar water and carboy water were averaged across the experiment, and an ANOVA was conducted on these estimates of the average conditions in each jar.

In Experiment 2, the difference in shell area at settlement between the two pH levels was analyzed using a Student's t-test. Juvenile shell growth rate was analyzed using an ANOVA with jar means as replicates, and larval rearing pH level and juvenile pH level as the main effects. The pH and alkalinity data were analyzed using separate Student's t-tests, one test for the larval phase of the experiment, and one test for the juvenile phase of the experiment. In all analyses, data fulfilled assumptions of normality and homogeneity of variance, tested using Shapiro-Wilks' and Bartlett's test, respectively, and thus untransformed data were used. Post-hoc Tukey-Kramer tests were applied at the α=0.05 level. The statistical software JMP (ver. 8.0.1, Statistical Analysis Software) was used for all analyses.

RESULTS

Water chemistry

Jar water and carboy water were combined for each jar for a time-averaged estimate of the pH within each jar through the duration of each experiment. pH levels in the treatments differed significantly from one another in each experiment (Experiment 1, ANOVA,

$F_{2,12}$=983.18, p<0.0001; Experiment 2, Student's t-test, Larval phase, t_{10}=41.54, p<0.0001; Juvenile phase, t_{22}=48.89, p<0.0001; Table 1). pH did not vary between boxes assigned to the same treatment in Experiment 1 (ANOVA, box[pH level], $F_{3,12}$=0.98, p=0.4362). Mean pH values differed by up to 0.07 unit from the nominal treatment levels of 8.0, 7.9, and 7.8. Such deviations could have arisen from incomplete equilibration between the seawater and the gas phase. pH also differed between seawater entering and exiting the culture jars due to accumulated effects of respiration over the two days separating water changes (mean oscillation 0.08 unit). Alkalinity did not differ between the pH 8.0 and 7.9 treatments in Experiment 1, but was significantly higher by 11–15 μmol/kg_{sw} in the pH 7.8 treatment (ANOVA, $F_{2,12}$=63.44, p<0.0001; Table 1). Alkalinity varied between boxes assigned to the same treatment in Experiment 1 (ANOVA, box[pH level], $F_{3,12}$=13.43, p=0.0004). In Experiment 2, alkalinity did not differ between the pH levels in either the larval or the juvenile phase of the experiment (Student's t-test, Larval phase, t_{10}=-0.33, p=0.7466; Juvenile phase, t_{22}=1.02, p=0.3185; Table 1).

Experiment 1

Reduced-pH seawater significantly influenced larval shell growth rate at day 9 post-larval release (ANOVA, $F_{2,12}$=5.12, p=0.0246; Fig. 2a). Larvae reared in the lowest pH exhibited 15% decreased shell growth rate compared to larvae raised in control pH. Larval shell growth rate did not differ among cohorts (ANOVA, $F_{3,12}$=0.019, p=0.9963). The effect of pH level on larval shell growth rate was not dependent on the cohort (ANOVA, pH level*larval cohort, $F_{6,12}$=1.04, p=0.4493), nor did the response vary between boxes assigned to the same treatment (ANOVA, box[pH level], $F_{3,12}$=0.60, p=0.6280).

Shell area at settlement varied significantly with pH level (ANOVA, $F_{2,12}$=6.15, p=0.0145; Fig. 2b); larvae reared in pH 7.8 conditions were 7% smaller at settlement than larvae

in control pH conditions. Shell area at settlement did not vary among larval cohorts (ANOVA, $F_{3,12}$=0.021, p=0.9958). The effect of pH level on shell area at settlement was not dependent on the larval cohort (ANOVA, pH level*larval cohort, $F_{6,12}$=0.134, p=0.9892), nor did the response vary between boxes assigned to the same treatment (ANOVA, box[pH level], $F_{3,12}$=1.49, p=0.2662).

Juvenile shell growth rates differed among pH levels at day 7 post-settlement (ANOVA, $F_{2,12}$=29.28, p<0.0001; Fig. 2c), with juveniles reared in pH 7.8 seawater exhibiting a 41% decrease in shell growth rates compared to juveniles in control conditions. Such declines were greater by a factor of 3 compared to those observed for larvae, representing the largest impacts observed in the study. Juvenile shell growth rates did not vary among larval cohorts (ANOVA, $F_{3,12}$=2.42, p=0.1163). The influence of pH level on juvenile shell growth rate did not vary among larval cohorts (ANOVA, pH level*larval cohort, $F_{6,12}$=0.77, p=0.6104), but the response varied between boxes assigned to the same treatment (ANOVA, box[pH level], $F_{3,12}$=10.01, p=0.0014). Since water chemistry did not differ between boxes, this effect might have been caused by other minor environmental differences between boxes (e.g., water temperature).

To explore the subsequent persistence of effects into early juvenile life, metamorphosed individuals were transferred from the culture conditions to a common garden 7 days after settlement, and shell growth rate was reassessed after 45 additional days. Juveniles that had been reared in pH 7.8 seawater as larvae and for the first 7 days following settlement exhibited a 29% reduction in shell growth rate over this period relative to juveniles that had been reared under control conditions throughout early life (ANOVA, $F_{2,12}$=13.94, p=0.0007; Fig. 3). Juvenile shell growth rate of individuals in the common garden did not differ among larval cohorts (ANOVA, $F_{3,12}$=2.33, p=0.1263). The response of juvenile shell growth rate to pH level was not dependent

on the larval cohort (ANOVA, pH level*larval cohort, $F_{6,12}$=0.81, p=0.5816), but the response varied between boxes assigned to the same treatment (ANOVA, box[pH level], $F_{3,12}$=6.64, p=0.0068).

Experiment 2

This experiment was performed to determine at what stage in the life cycle the magnified impacts of acidified seawater on juvenile shell growth rates originated (i.e., the 41% decreases of Fig. 2c). Larvae from the two pH treatments did not differ in shell area at settlement (Student's t-test, t_{22}=0.149, p=0.8827), but subsequent shell growth rates of juveniles depended on the conditions that they experienced as larvae (ANOVA, larval pH level, $F_{1,21}$=16.57, p=0.0005; Fig. 4), consistent with a larval carry-over effect (Fig. 1). On day 7 post-settlement, juvenile shell growth rate was substantially reduced (by up to 26%) in treatments where larvae were reared under low pH (7.8→7.8 and 7.8→8.0 treatments), compared to growth of individuals raised entirely under control pH (8.0→8.0 treatment). There was no indication that individuals switched to different seawater pH conditions (8.0→7.8 and 7.8→8.0 treatments) were disproportionately impacted. Individuals exposed as juveniles to low pH (7.8→7.8 and 8.0→7.8 treatments) also did not exhibit a consistent trend of reduced growth. In other words, juvenile shell growth rate depended on the pH treatment to which the oysters were exposed as larvae, but not the pH treatment to which the juveniles were exposed for 7 days post-settlement (ANOVA, juvenile pH level, $F_{1,20}$=0.097, p=0.337; larval pH level*juvenile pH level, $F_{1,20}$=0.022, p=0.884). Although not quantified directly, juvenile mortality during the week following settlement appeared to be very low (Hettinger et al., pers. observ.). Therefore, the smaller size of juveniles raised as larvae under elevated pCO_2 likely reflected a reduced growth rate, rather than differential mortality of larger juveniles.

DISCUSSION

A large fraction of terrestrial and aquatic animal taxa have complex life cycles, including over 70% of benthic marine species (Mileikovsky 1971). As revealed in the present study, this attribute can complicate assessment of species' vulnerabilities to environmental change. In the Olympia oyster, effects of ocean acidification were transmitted strongly across life stages and led to pronounced impacts that did not fully manifest until after larvae settled and underwent metamorphosis. This outcome has at least two implications for interpreting the role of a "weak link" in the response of organisms to environmental stress. First, such approaches neglect life-stage connections that can alter overall impacts. Based on the data of Figs. 2c and 4, if effects on larvae and juveniles had been assayed separately, and then simply summed, one would have predicted an order 10% decline in growth over the course of early life (i.e., 7% size reduction at settlement in the pH 7.8 treatments of Experiment 1, coupled with minor reductions in the pH 8.0→7.8 versus 8.0→8.0 case of Experiment 2). Such estimated impacts are a factor of 2–4 below those observed when carry-over effects were considered explicitly. Second, focusing on a perceived weak link can misattribute windows of susceptibility to incorrect stages. During Experiment 1, juvenile growth rate in the pH 7.8 treatment was reduced 41% relative to control conditions, compared to a 7% reduction at the time of settlement. Such a pattern suggests a high sensitivity of juveniles to elevated seawater acidity, and a reduced sensitivity of larvae. However, accounting for carry-over effects leads to exactly the opposite conclusion.

Cause for reductions in shell growth during early life: A matter of energetics

The modest reductions in *O. lurida* shell growth during the larval phase, and the magnified declines in shell growth rate during the juvenile phase, may both be tied to energetics. Mineral accretion, maintenance of internal acid-base balance, production of somatic and

reproductive tissue, and behaviors such as swimming and feeding, all incur metabolic costs (Pörtner et al. 2004). Some, but not all, invertebrates experience higher metabolic rates in response to elevated pCO_2 concentrations (Pörtner et al. 2004, Beniash et al. 2010, Stumpp et al. 2011). The process of calcification can be a major component of the energy budget (Palmer 1992, Cohen and Holcomb 2009), and energetic costs of shell building may increase as carbonate ion concentrations decline. The response of calcifying species to ocean acidification is influenced by their degree of control over fluid pH at the site of calcification (Ries 2011). Maintenance of a specific calcifying fluid pH, as occurs in some bivalve species (Crenshaw 1972), might help sustain calcification in elevated-pCO_2 conditions, but may also add energetic costs that ultimately reduce growth rates (Wood et al. 2008). Although in our study conditions never became undersaturated with respect to calcite or aragonite, the drop in pH levels could have been enough to create a more challenging shell-building environment. If conditions were to fall below a saturation state of 1, the effect would presumably become even larger.

Such energetic costs may have contributed to the substantial larval carry-over effect observed in *O. lurida*. Oyster larvae reared in acidified seawater may have initiated metamorphosis in an energy depleted state, and metamorphosis is an energetically expensive process. Indeed, even in normal seawater, over 60% of the energy stores accumulated by Chilean oyster larvae are expended during metamorphosis (Videla et al. 1998). Although larvae of some species may offset negative effects of acidified seawater on their energy budgets by extending the planktonic larval duration to provide more time to build reserves before metamorphosis (Talmage and Gobler 2009), an extended pelagic period may also increase exposure to predation in the plankton (Rumrill 1990).

Demographic implications

Carry-over impacts of ocean acidification may have relevance for understanding responses of a variety of bivalves to changes in seawater chemistry, and have clear potential implications for population dynamics of the Olympia oyster. Even though the pH levels tested are unlikely to occur continuously for several decades, they already arise episodically during certain seasons at our field sites. Size-specific mortality rates have not yet been determined for *O. lurida*, but completing metamorphosis at a below-average size risks increased mortality during a vulnerable period for many benthic species (Gosselin and Qian 1997, Gaylord et al. 2011). Furthermore, because early life stages often act as population bottlenecks, impacts following settlement could cascade to influence adult populations. Negative effects of ocean acidification persisted in *O. lurida* for over 7 weeks into juvenile life, and although reproduction in this species occurs at approximately 6 months of age, studies in other terrestrial and aquatic taxa have demonstrated that an individual's size at metamorphosis can correlate with its future adult fecundity and fitness (Semlitsch et al. 1988, Berven 1990, Marshall and Keough 2008).

Carry-over effects and global environmental change

Our results emphasize that effects of climate change can be transferred, and sometimes magnified, across life stages in organisms with complex life histories. Understanding the factors that modify the relative strengths of such carry-over effects is an important, and largely unexplored, area of research. Previous work suggests that the ability of early life stages to tolerate environmental stresses may be strongly dependent on their initial size and/or physiological and nutritional condition. For example, in some amphibians, larvae from large eggs may be more tolerant of environmental stresses tied to high population density, and reach a larger size at metamorphosis than larvae derived from small eggs (Berven and Chadra 1988). In our study system, it is possible that oyster larvae maintained under high levels of planktonic food

would attain nutritional reserves that would buffer them against certain stresses of ocean acidification, as suggested by recent work on other bivalves (e.g., Melzner et al. 2011). Experiments are underway to determine whether food concentrations available to Olympia oyster larvae could modify the strength of negative carry-over effects (see also, Emlet and Sadro 2006, Dmitriew and Rowe 2011). In addition, the environment that juveniles experience following metamorphosis may play a role in determining the intensity and duration of carry-over effects. Under favorable conditions, compensatory growth after metamorphosis might rapidly diminish carry-over effects (Morey and Reznick 2001, Emlet and Sadro 2006). In contrast, stressful environmental conditions following metamorphosis might intensify them (Dmitriew and Rowe 2011). Olympia oyster larvae settle at different shore levels and locations along estuarine gradients, and can therefore exhibit variation in juvenile growth as a function of temperature, desiccation, and phytoplankton availability (Kimbro et al. 2009). A growing body of work points to the potential interactive effects of multiple environmental stressors on natural populations (e.g., Crain et al. 2008). Thus, negative carry-over effects of ocean acidification might be exacerbated in juvenile oysters that are also challenged by high temperatures, pollution, or other stresses. Finally, taxa with complex life histories possess diverse modes of development. For example, marine fish and invertebrate species include those with both feeding and non-feeding larvae. Species with these contrasting modes of development will likely respond differently to stresses like ocean acidification, but there are few empirical data with which to address this hypothesis (Dupont et al. 2010, E. S. L. Kuo et al., unpublished data). Predicting impacts of global environmental change on species with complex life histories will benefit from explicitly addressing the factors that lead to and modify the strength of carry-over effects.

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565 **APPENDIX A**

566 A photograph of a juvenile *Ostrea lurida*. (Ecological Archives Exxx-xxx-A1).

567

TABLE 1. A table of seawater properties during each experiment of this study (± standard deviation computed from averages of the jar replicates). Temperature, salinity, alkalinity, and pH_{NBS} are measured values, and pCO_{2calc}, $\Omega_{calcite}$, and $\Omega_{aragonite}$ are values calculated using the carbonate system analysis software program, CO2SYS (Lewis and Wallace 1998). For Experiment 2, the top numbers for each parameter are data from the larval phase of the experiment when 6 jars/pH level were used, and the bottom numbers are data from the juvenile phase of the experiment, after settled juveniles were placed into new pH treatments (12 jars/pH level). The superscripted letters before the alkalinity and pH_{NBS} values for Experiment 1 indicate results of Tukey-Kramer post-hoc tests; shared letters indicate values that did not differ significantly between treatments ($p>0.05$). For Experiment 2, significant or non-significant differences at the $\alpha=0.05$ level are indicated with an * or NS, respectively (Student's t-test).

580

	Nominal pH treatment		
	8.0	*7.9*	*7.8*
Experiment 1			
Temperature (°C)	20 ± 0.605	20 ± 0.439	20 ± 0.58
Salinity (psu)	33.6 ± 0.16	33.6 ± 0.16	33.6 ± 0.16
Alkalinity (μmol/kg_{sw})	^{A}2220 ± 3.97	^{A}2223 ± 6.25	^{B}2235 ± 3.11
pH_{NBS}	^{A}7.93 ± 0.011	^{B}7.86 ± 0.007	^{C}7.73 ± 0.006
pCO_{2calc}	739 ± 51	933 ± 29	1355 ± 37
$\Omega_{calcite}$	2.36 ± 0.053	2.01 ± 0.03	1.55 ± 0.019
$\Omega_{aragonite}$	1.62 ± 0.037	1.39 ± 0.02	1.07 ± 0.013
Experiment 2			
Temperature (°C)	20 ± 0.198 20 ± 0.224	----------	20 ± 0.242 20 ± 0.187
Salinity (psu)	33.4 ± 0.23 34.0 ± 0.23	----------	33.4 ± 0.23 34.0 ± 0.23
Alkalinity (μmol/kg_{sw})	2231 ± 16.1 2216 ± 13.6	----------	^{NS}2233 ± 9.8 ^{NS}2215 ± 13.9
pH_{NBS}	7.96 ± 0.008 7.95 ± 0.007	----------	*7.77 ± 0.004 *7.79 ± 0.007
pCO_{2calc}	714 ± 11 757 ± 20	----------	1161 ± 11 1083 ± 21
$\Omega_{calcite}$	2.51 ± 0.048 2.43 ± 0.036	----------	1.7 ± 0.014 1.74 ± 0.027
$\Omega_{aragonite}$	1.73 ± 0.033 1.67 ± 0.025	----------	1.17 ± 0.01 1.2 ± 0.018

581

582

583 FIGURE LEGENDS

584 FIG. 1. Design and hypotheses for Experiment 2, directed at determining the origin of ocean
585 acidification effects on *Ostrea lurida* juvenile shell growth. (A) The cylinders represent one jar
586 in a given treatment, with the associated number indicating the pH level in which larvae or
587 juveniles were reared. Larvae were raised in either control (pH 8.0) or acidified (pH 7.8)
588 conditions. Immediately following settlement, half of the new juveniles from each jar were
589 transferred to the alternate pH condition (8.0→7.8 or 7.8→8.0), while half were returned to the
590 treatment in which they originated (8.0→8.0 or 7.8→7.8). (B) Graphical representation of the
591 three hypothetical responses of juvenile shell growth to the four larval-juvenile pH treatments.
592 FIG. 2. Effect of ocean acidification on larval and juvenile *Ostrea lurida* during Experiment 1.
593 Data are (A) larval shell growth rates in *Ostrea lurida* on day 9 post-larval release, (B) shell area
594 at the time of settlement, and (C) juvenile shell growth rates on day 7 post-settlement. Values are
595 means across all jar replicates of a given treatment + 1 SE. Shared letters above bars indicate
596 responses that did not differ significantly (Tukey-Kramer, $p>0.05$).
597 FIG. 3. Effect of ocean acidification on *Ostrea lurida* juvenile shell growth rate at day 52 post-
598 settlement, 45 of which were spent in a common environment, during Experiment 1. Values are
599 means across all jar replicates of a given treatment + 1 SE. Different letters above bars indicate
600 responses that differed significantly (Tukey-Kramer, $p<0.05$).
601 FIG. 4. Effects of four larval-juvenile ocean acidification treatments on *Ostrea lurida* juvenile
602 shell growth rate at day 7 post-settlement during Experiment 2. The upper row of values under
603 each bar indicates the pH in which larvae were reared through settlement, and the lower row
604 indicates the pH in which juveniles were reared until 7 days post-settlement. Values are means
605 across all jar replicates of a given treatment + 1 SE.

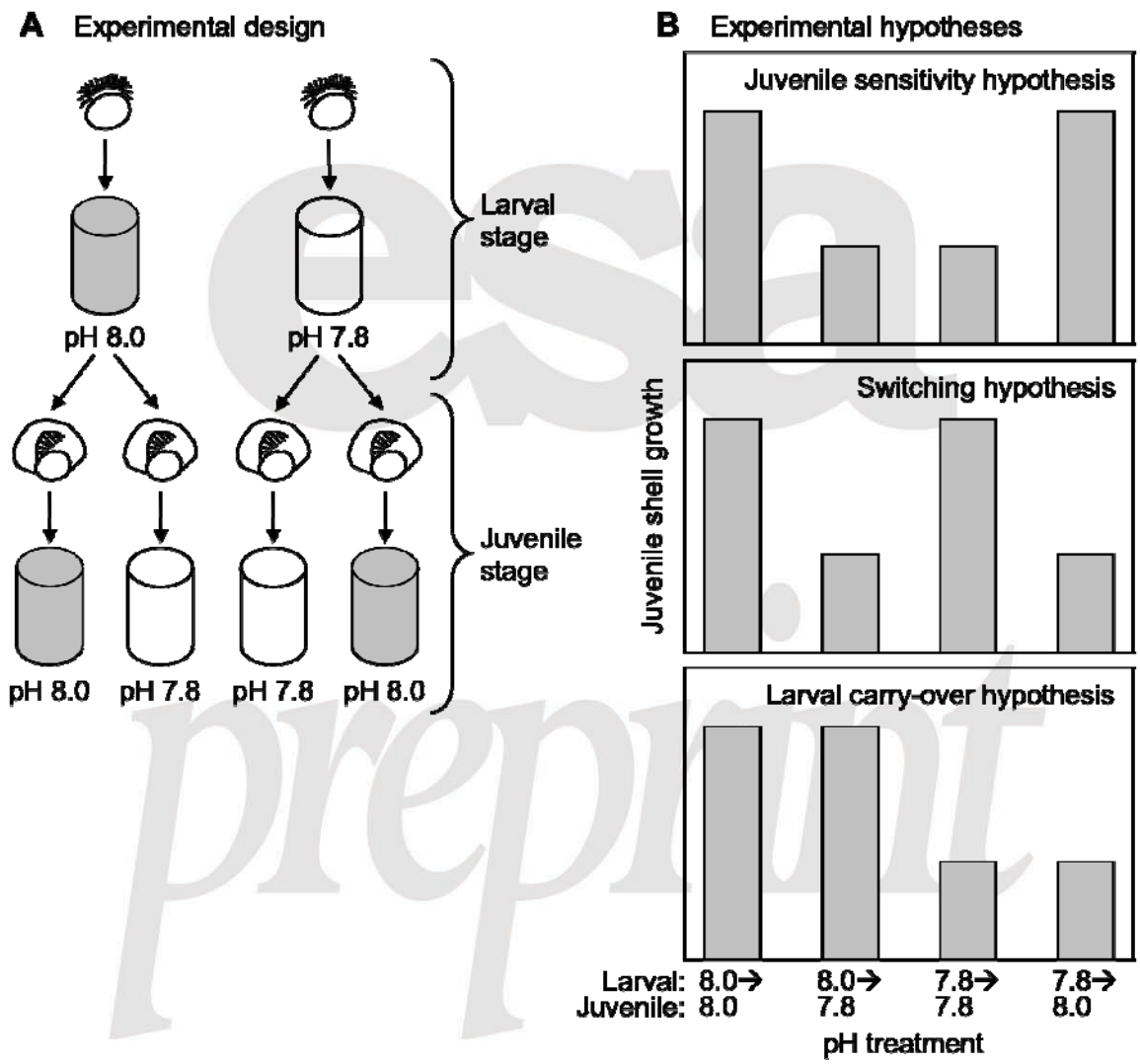

Figure 1.

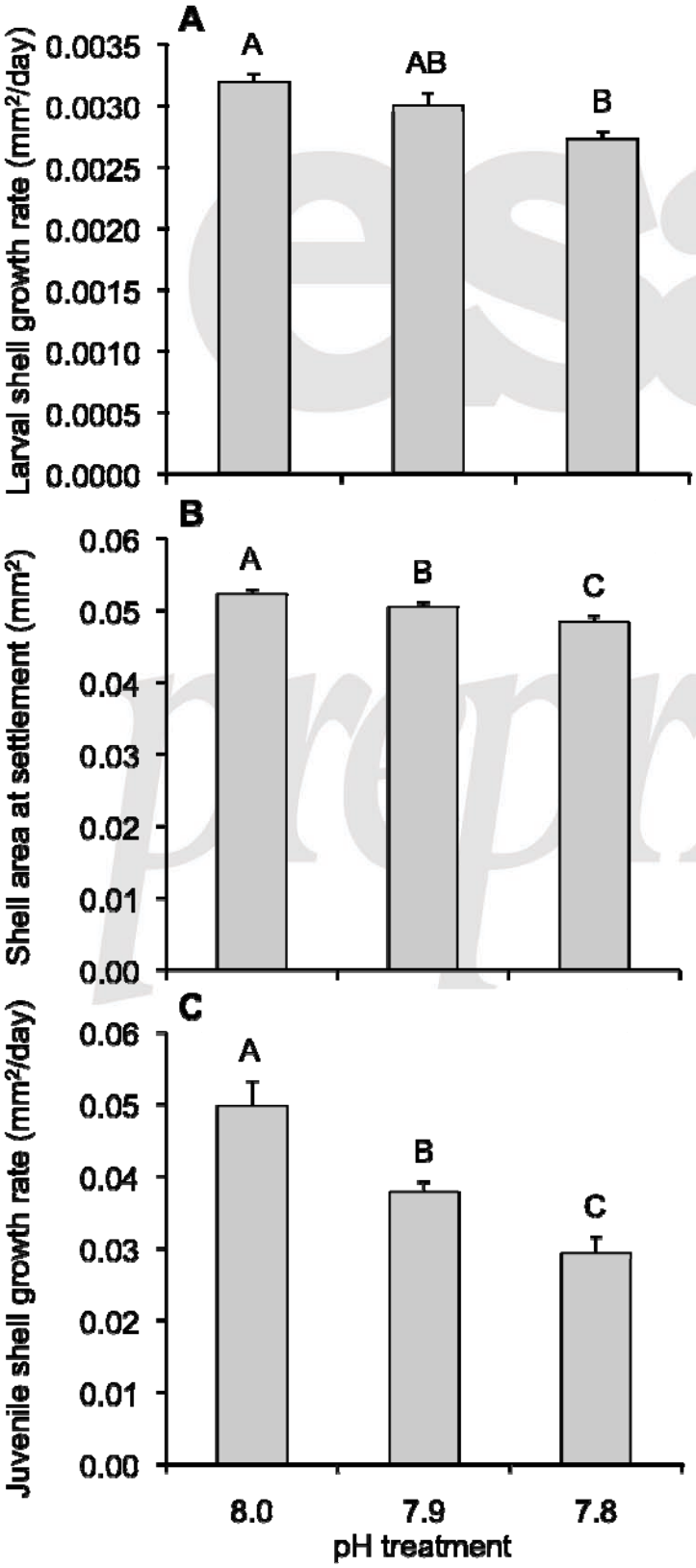

Figure 2.

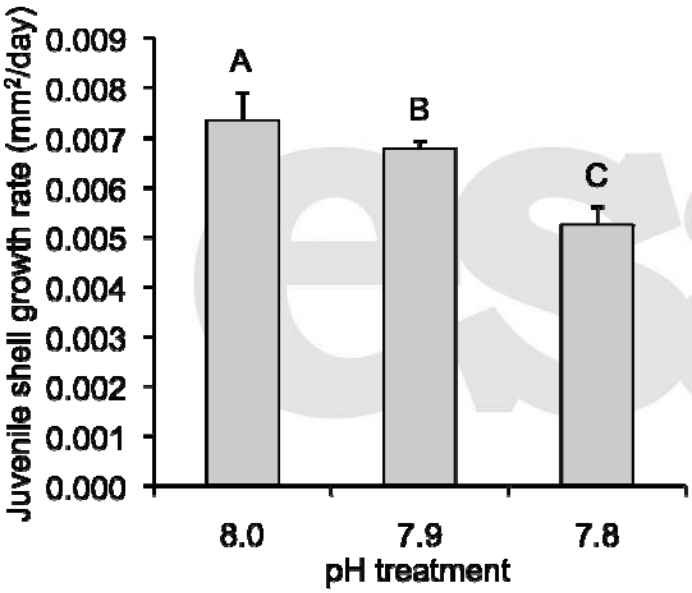

Figure 3.

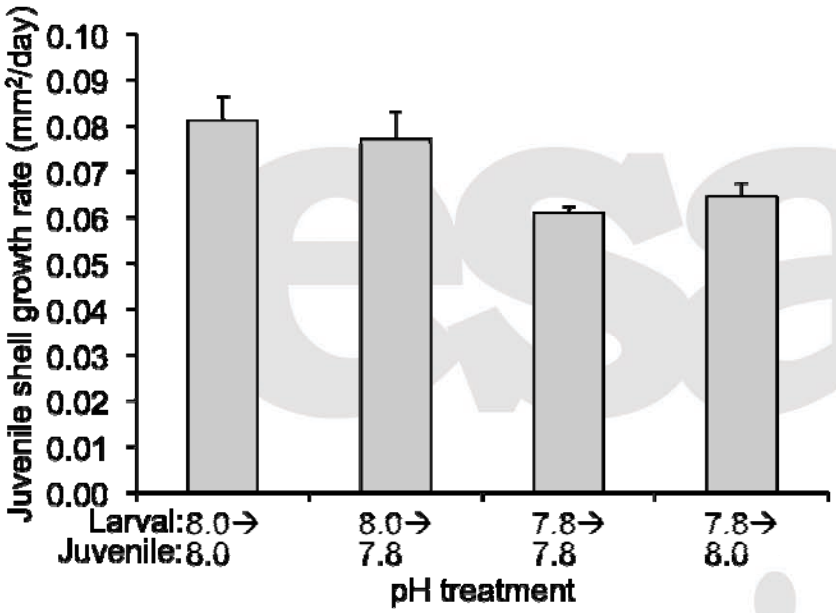

Figure 4.